

CLAIMS

We claim:

1. A method of scalable purification of adenoviral preparations comprising the steps of:
 - a) culturing host cells comprising adenovirus;
 - b) obtaining supernatants from the host cells of step a);
 - c) applying said supernatants to a centrifugal apparatus comprising a 50% w/v solution of non-ionic gradient;
 - d) applying centrifugal force to said supernatants such that the flow is continuous and directed from bottom-to-top;
 - e) separating the adenoviral particles according to their density; and
 - f) obtaining high-yield fractions comprising active adenoviral particles.
2. The method of claim 1, wherein said adenovirus is a human adenovirus.
3. The method of claim 2, wherein said human adenovirus is non-oncogenic.
4. The method of claim 2, wherein said human adenovirus is human adenovirus serotype-5.
5. The method of claim 1, wherein said adenovirus comprises heterologous DNA sequences.
6. The method of claim 5, wherein the heterologous DNA sequence comprises a therapeutic gene.
7. The method of claim 1, where in the gradient comprises Nycodenz®.
8. The method of claim 7, wherein the fraction is obtained from an isodense point of about 55% to about 35% Nycodenz®.
9. The method of claim 7, wherein the fraction is obtained from an isodense point of about 45% Nycodenz®.
10. The method of claim 1, wherein the continuously flowing liquid comprises a buffered salt solution.
11. The method of claim 1, wherein the continuously flowing liquid comprises an adenovirus-laden cell culture supernatant.
12. The method of claim 1, wherein the flow rate of step d) is about 40 ml/min.
13. The method of claim 1, wherein the fractions are collected using air pressure.
14. The method of claim 1, wherein the fractions are collected using water pressure.

15. The method of claim 13, wherein the collection of the fractions is aided by use of a pumping mechanism.
16. The method of claim 14, wherein the collection of fractions is aided by use of a pumping mechanism.
17. The method of claim 15 or 16, wherein the pumping mechanism used is a peristaltic pump.
18. A method of preparing a gradient for continuous flow ultracentrifugation comprising:
 - a) filling a rotor with buffer through lines leading into the top and bottom of the rotor;
 - b) accelerating the rotor while maintaining a buffer flow rate of about 200 ml/min and increasing the buffer flow to about 300 ml/min at a speed of at least 10,000 rpm;
 - c) shifting the direction of flow between top-to-bottom and bottom-to-top at least once;
 - d) loading a density gradient material into the rotor at rest;
 - e) gradually accelerating the rotor while maintaining a buffer flow rate of about 200 ml/min;
 - f) switching the direction of flow to bottom-to-top at about 3200 rpm and reducing the flow rate to about 80 ml/min;
 - g) reducing the flow rate to about 40 ml/min at about 40,500 rpm; and
 - h) forming a gradient.
19. A gradient formed by the method of claim 18.